in rats. In the fear conditioning paradigm animals learn to associate a neutral environment (context, the training chamber, CS) with an aversive experience (an electrical foot-shock, US). During re-exposure to the training chamber, animals express a freezing behaviour, which is taken as a direct measure of the fear-related memory [*Pavlov J. Biol. Sci.,* 15, 177-182, 1980]. The neuroanatomy of contextual fear conditioning has been thoroughly investigated and several studies have demonstrated that the hippocampus and amygdala are necessary for the formation of this memory [*Hippocampus,* 11, 8-17, 2001; *J. Neurosci.,* 19, 1106-1114, 1999; *Behav. Neurosci.,* 106, 274-285, 1992].

Animals and Drugs

Adult male Sprague-Dawley rats (weighing 250-300 g at time of training) from Charles River Laboratories, housed two per cage under a 12 h light/dark cycle, were used. Food and water were available ad libitum. Rats were used 1 week after arrival. The compound was dissolved in 10% HPbetaCD and injected subcutaneously. The drug was administered in a volume of 2.5 ml/kg.

Apparatus

Training and testing were conducted in a soundproof chamber (30×20×40 cm) housed in an isolated room and connected to a ventilation system. Illumination was provided by a white light (60 Watt). The floor of the chamber consisted of a metal grid attached to an electric shock generator. Prior to training and testing, the chamber was cleaned with a 70% ethanol solution. A video camera allowed for behavioral observations and recording of the training session for off-line analysis.

Acquisition and Retention Test

During the acquisition animals were allowed to freely explore the novel environment for a 1 min habituation period, which co-terminated with one inescapable foot-shock (unconditioned stimulus, US) through the electrifiable grid floor. The foot shock had a duration of 2 s and an intensity of 0.75 mA. Animals remained in the conditioning chamber for another 60 s after the US. Freezing behaviour was scored during the first 58 s (pre-shock acquisition; experimenter blinded to groups) to determine baseline-freezing responses to the context. At the end of the acquisition animals were gently removed and placed into their home cages. After 24 h the same animals were reintroduced into the training context (fear conditioning chamber) and a 2 min retention test was performed. During this period no foot shocks were applied. Freezing behaviour was scored during the whole test period with the experimenter blinded to groups and presented as percent of total test period.

Results and Discussion

Effect of the Compound on Contextual Fear Cognition in Rats

The effect of the compound on contextual fear conditioning in rats was studied (i) on acquisition (drug applied before acquisition, FIG. **20**), (ii) on memory recall (drug applied before test, FIG. **22**) and (iii) on consolidation (drug applied immediately after the acquisition, FIG. **21**). In the first set of experiments, the compound (1, 5 and 10 mg/kg) was administered 1 h prior to the acquisition session. FIG. **20** depicts the acquisition of freezing behavior during training (58 s prior to the food shock) and the retention test **24** after. The following findings were observed:

- The compound does not affect baseline freezing behaviour before the presentation of the foot shock at any dose tested.
- The compound at 5 mg/kg has a tendency to increase the time spent freezing during the retention test, 24 h after the acquisition (39.24±13.76%, n=6, versus 24.30±4.40%, n=16, in the vehicle-treated animals).
- The compound at 10 mg/kg significantly increases the time spent freezing during the retention test, 24 h after the acquisition (52.15±5.68%, n=10, versus 24.30±4.40%, n=16, in the vehicle-treated animals, $p<0.01$).

The fear conditioning model, as described in FIG. **20**, is a standard procedure described in the literature for the investigation of learning and memory. In order to further elucidate the acute effects of this drug on memory recall, the compound (5, 10 and 20 mg/kg) was applied 1 h prior to the retention test. It was observed that the compound inhibits the expression of freezing behaviour at 5 mg/kg during the memory test (12.86±3.57%, n=9, versus 33.61±4.29%, n=13, in the vehicle-treated animals, $p<0.05$) (FIG. **22**).

As described above, the compound by itself does not affect baseline freezing behaviour before the onset of US (FIG. **20**), thus the most plausible hypothesis is that the observed effect in FIG. **22** is due to an anxiolytic effect. The conditioned memory is assessed via freezing behaviour, a response that is reduced by compounds with potential anxiolytic effects. This experiment demonstrates that the compound given acutely before memory recall has anxiolytic efficacy, it is therefore unlikely that increased freezing shown in FIG. **20** is due to an anxiogenic effect of the compound.

In order to strengthen that the compound is not anxiogenic but bears pro-cognitive potential, the compound was administered at 5, 10 and 20 mg/kg after the acquisition session. Consequently, in this set of experiments, the compound was onboard neither during the acquisition nor throughout the retention test. Here, it was observed that the compound at 5 mg/kg significantly enhances the time spent freezing during the retention test, 24 h after the acquisition session (45.58±4.50%, n=8, versus 25.26±3.57%, n=19, in the vehicle-treated animals, $p<0.05$). The percentage of time spent freezing during the context re-exposure has been described as a measure of a fear-related memory [*Pavlov J. Biol. Sci,* 15, 177-182, 1980], which is enhanced in compound-treated rats when compared to vehicle-treated animals (FIGS. **20** and **21**). Taken together, the data show that the compound enhances contextual memory.

The invention claimed is:

1. A method of alleviating a symptom or complication of depression or major depressive disorder, or delaying progression of depression or major depressive disorder, comprising:

administering to a patient in need thereof a pharmaceutical composition comprising a hydrobromide salt of a 1-[2-(2,4-dimethylphenylsulfanyl)-phenyl]piperazine selected from the group consisting of

1-[2-(2,4-dimethylphenylsulfanyl)-phenyl]piperazine hydrobromide salt alpha form,

1-[2-(2,4-dimethylphenylsulfanyl)-phenyl]piperazine hydrobromide salt beta form,

1-[2-(2,4-dimethylphenylsulfanyl)-phenyl]piperazine hydrobromide salt gamma form,

1-[2-(2,4-dimethylphenylsulfanyl)-phenyl]piperazine hydrobromide salt hemihydrate,

1-[2-(2,4-dimethylphenylsulfanyl)-phenyl]piperazine hydrobromide salt ethyl acetate solvate, and mixtures thereof,

wherein said method alleviates a symptom or complication of depression or major depressive disorder, or delays the progression of depression or major depressive disorder, in said patient.